

The Correlation between CD64 Leukocyte, Immature Granulocyte and resepsin with Procalcitonin in Bacterial Sepsis Patient

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The Correlation between leucocyte CD64, Immature Granulocyte and Presepsin with Procalcitonin in Bacterial Sepsis Patient



Citra Novita,¹ Yetti Hernaningsih,^{1*} Puspa Wardhani,¹ Anna Surgean Veterini²

ABSTRACT

Background: Sepsis is a critical emergency that causes morbidity and mortality worldwide. The latest sepsis diagnosis is made by using quick Sepsis-Related Organ Failure Assessment (qSOFA). Cluster of Differentiation 64 (CD64) is a surface antigen leukocyte that is deregulated during infection and sepsis. The percentage of immature granulocyte (IG) could rise in patients with infection and sepsis, mainly in severe circumstances. Procalcitonin (PCT) is a calcitonin prohormone that increases in sepsis and is already known as a bacterial infection marker. Presepsin (CD14) is a glycoprotein that is known to increase in bacterial infection. This study aimed to determine the correlation of leucocyte CD64, IG, and presepsin with PCT in bacterial sepsis patients. **Method:** This cross-sectional study was performed from June to September 2018 at Dr. Soetomo General Academic Hospital. Twenty-five patients who met the qSOFA criteria with positive bacterial blood cultures were

included. All samples underwent examinations of leucocyte CD64, IG, presepsin, and PCT. The correlation of leucocyte CD64, IG and presepsin with PCT was analyzed using Spearman correlation.

Results: The samples comprised 17 males (68.0%) and 8 females (32.0%). The mean age was 51.24 ± 14.85 years. The mean \pm SD of leucocyte CD64 was $6.95 \pm 2.13\%$, the median (min-max) of IG, presepsin and PCT was 3.67 ($0.33-17.33$), $2,641$ ($487-20,000$) pg/mL and 5.96 ($0.39-181.5$) ng/mL respectively. There was no correlation between leucocyte CD64 with PCT ($p = 0.281$). There was a significant correlation between IG and presepsin with PCT ($p < 0.0001$).

Conclusions: Presepsin and IG can be used as alternative bacterial sepsis markers that are supported by other examinations. leucocyte CD64 still needs to be studied further before it can be used as a bacterial sepsis marker.

Keywords: Bacterial sepsis, leucocyte CD64, immature granulocyte, presepsin, procalcitonin.

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INTRODUCTION

Sepsis is a serious problem and a critical emergency, which causes morbidity and mortality around the world. Therefore, early diagnosis is required immediately.¹ The prevalence of severe sepsis in America is more than 700,000 cases a year with mortality in an average of 30%.² The incidence of sepsis in Dr. Soetomo General Academic Hospital increased in the last two years. The incidence in 2003 was 2,446 cases and 3,060 cases in 2014. Sepsis cases mostly affect patients aged 45-64. Deaths caused by sepsis were 1,653 cases of 3,060 cases (54.0%) in 2014 and 1,487 of 2,446 cases (60.8%) in 2013.³

The Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM) suggest a new definition called quick Sepsis-Related Organ Failure Assessment (SOFA). This new proposal defines sepsis as life-threatening organ dysfunction, caused by host dysregulation due to infection. Quick SOFA (qSOFA) is based on two of three organ dysfunction signs, which are altered mental status (Glasgow Coma Scale ≤ 13), systolic blood pressure ≤ 100 mmHg, and respiratory rate ≤ 22 times per minute.⁴

Early clinical diagnosis often causes difficulties due to minimal signs of infection. Blood culture is the gold standard for detecting pathogens in sepsis patients, but it takes a long time to get the result of pathogens identification, so the death rate of sepsis patients is very high due to late diagnosis. Several biomarkers have been designated for the diagnosis of sepsis, yet the most reliable biomarkers for accurate diagnosis of sepsis patients are still controversial.¹

Cluster of Differentiation 64 (CD64) is a leukocyte surface antigen that is regulated during infection and sepsis. The CD64 expression can increase clearly at the beginning of sepsis and is found to be a diagnostic marker in adults and children. The study conducted by Hassuna et al. concluded that CD64 expression increased significantly in sepsis with positive culture results.¹

Immature granulocytes (IG) consisting of promyelocytes, myelocytes, and metamyelocytes are useful markers for predicting infection and its severity in critically ill patients. The percentage of IG can increase in patients with infection and sepsis,

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especially in severe conditions. A study of critical illness suggested that calculating the percentage of IG was better at predicting sepsis with suspected or proven infection than CRP.⁵

Procalcitonin (PCT) is a prohormone of calci-tonin, first described as a specific marker of bacterial infection in 1990. It increases during sepsis and is known as the marker of infectious diseases. It can reach 1000 ng / mL during severe sepsis and septic shock.⁶ PCT levels of 0.5 ng / mL increase suspicion towards sepsis, but usually these patients have higher PCT levels.⁵

Presepsin is a glycoprotein that is encoded by genes and has a role as a component of the innate immune system. Presepsin levels were significantly higher in sepsis patients compared to SIRS or healthy individuals. Presepsin can be used to determine the diagnosis, prognosis and monitor the course of sepsis.⁷

PCT examination, as a reference sepsis biomarker, has been widely used including in Indonesia. Much research has been done on sepsis in Dr. Soetomo General Academic Hospital, and the most frequently tested biomarker is PCT. leucocyte CD64 and presepsin are new parameters that can potentially be used as biomarkers of sepsis. leucocyte CD64 examination and presepsin are said to have a higher sensitivity and specificity than PCT. IG has the advantage of being more efficient in terms of cost and feasibility compared to PCT. The purpose of this study was to analyze the correlation of leucocyte CD64, IG and presepsin with PCT in bacterial sepsis patients to provide another screening alternative in diagnosing sepsis.

METHODS

This was an observational analytical cross-sectional study. Samples were patients who met the qSOFA criteria with positive bacterial blood culture. Sample collection was conducted from June to September 2018 in the Intensive Observation Room (ROI), Intensive Care Unit (ICU), Emergency unit, and inpatient ward of Dr. Soetomo General Academic Hospital, Surabaya Indonesia. The sampling method was consecutive sampling. All subjects who participated in this study were willing to take part in the study by signing an informed consent.

The inclusion criteria were adult patients (≥ 18 years) admitted to Dr. Soetomo General Academic Hospital within 48 hours who fulfilled the qSOFA criteria and had positive bacterial blood culture results. The exclusion criteria were patients with a history of liver abnormalities, hepatitis, diabetes mellitus,

malignancy, HIV infection and patients with immunosuppressant therapy.

Patients diagnosed with sepsis based on qSOFA and positive blood cultures immediately underwent leucocyte CD64, IG, presepsin, and PCT examination. Examination of CD64 and IG were taken from whole blood samples (EDTA). Presepsin examination was taken from the plasma, and PCT was taken from the serum. The results of blood culture were secondary data released by the microbiology laboratory of Dr. Soetomo General Academic Hospital. The percentage of leucocyte CD64 was examined using the immunoflowcytometry method using BD FACSCalibur with a detection limit of 0 - 100%. The percentage of IG was checked manually by making a peripheral blood smear with a range of readings of 0-100%. The percentage of IG was obtained based on the average reading of 3 competent people. Presepsin was examined by the Chemiluminescence Immunoassay (CLIA) method using the Pathfast tool with a detection limit of 20 - 20,000 pg / mL. The PCT level was examined by the CLIA method using the ADVIA Centaur tool with a detection limit of 0.01 - 75 ng / mL. The ethics committee of Dr. Soetomo General Hospital Surabaya agreed and stated that this research was ethical.

Data were analyzed using the SPSS version 17.0 program using the Kolmogorov-Smirnov test to determine the distribution of the data. The Spearman correlation test was used in this study because the data were not normally distributed.

RESULT

Sample characteristics

The number of research subjects was 86 samples that met the qSOFA criteria. Seven samples were excluded because their culture results were not found. The number of patients with positive bacterial culture results was 25 (31.64%) out of 79 patients. Sample characteristics can be seen in Table 1.

The study samples underwent examinations of leucocyte CD64, IG, presepsin, and PCT. Blood cultures were secondary data with positive bacterial results. The characteristic of blood culture results is shown in Table 2.

Results of leucocyte CD64, IG, Presepsin, and Procalcitonin

The mean (\pm SD) of leucocyte CD64 was 6.95 (\pm 2.14). The median value of IG and presepsin were 3.67% and 2,641 pg/mL respectively. The median value of PCT was 5.96 ng / mL. The mean (\pm SD) of leucocyte CD64, median, minimum and maximum

values of IG, presepsin, and PCT in bacterial sepsis patients in this study can be seen in Table 3.

Correlation of leucocyte CD64, IG, and presepsin with PCT

The Spearman correlation statistical test showed no correlation between leucocyte CD64 and PCT ($p = 0.281$; $r = 0.224$). There was a significant positive correlation between IG and PCT ($p < 0.0001$; $r = 0.663$). Presepsin showed a significant positive correlation with PCT ($p < 0.0001$; $r = 0.695$) in bacterial sepsis patients. The correlation of leucocyte CD64, IG, and presepsin with PCT can be seen in Figure 1a, 1b, 1c.

Table 1 Characteristics of sample

Characteristics	N = 25	%	Median (min-max)	Mean (\pm SD)
Gender				
Males	17	68.0%		
Females	8	32.0%		
Room				
ICU	5	20.0%		
ROI	9	36.0%		
Emergency unit	7	28.0%		
Inpatient ward	4	16.0%		
Age (years)				51.24 (\pm 14.85)
Leukocyte count ($10^3/\text{mm}^3$)				17.44 (\pm 8.59)
Systolic blood pressure (mmHg)			100 (90-200)	
Respiratory rate (x/minute)			28 (20-40)	

SD: standard deviation

Table 2 Blood culture in bacterial sepsis patients

Gram (+) bacteria	N (%)	Gram (-) bacteria	N (%)
<i>S. haemolyticus</i>	5 (20.0%)	<i>K. pneumonia</i> *	4 (16.0%)
<i>S. aureus</i>	(12.0%)	<i>A. baumannii</i>	4 (16.0%)
<i>S. epidermidis</i>	3 (12.0%)	<i>E. coli</i>	2 (8.0%)
<i>S. hominis</i>	1 (4.0%)	<i>B. aeruginosa</i>	1 (4.0%)
<i>L. monocytogenes</i>	1 (4.0%)	<i>A. testosterone</i>	1 (4.0%)

*1 isolate was *K. pneumonia* ESBL

Table 3 Examination results of leucocyte CD64, IG, presepsin, and PCT

Parameter	Mean (\pm SD)	Median (Min – max)
leucocyte CD64 (%)	6.95 (\pm 2.14)	
IG (%)		3.67 (0.33 – 17.33)
Presepsin (pg/mL)		2.641 (487 – 20.000)
PCT (ng/mL)		5.96 (0.39 – 181.5)

SD: standard deviation; CD: cluster of differentiation; IG: immature granulocyte; PCT: procalcitonin.

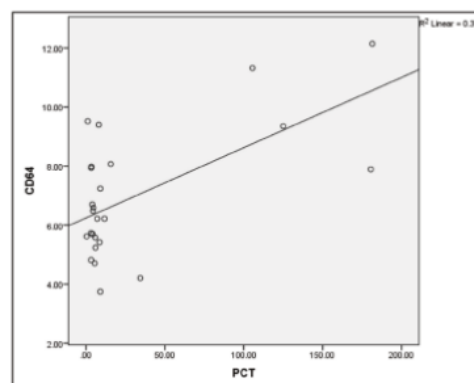


Figure 1a Correlation between leucocyte CD64 and PCT ($p = 0.281$; $r = 0.224$)

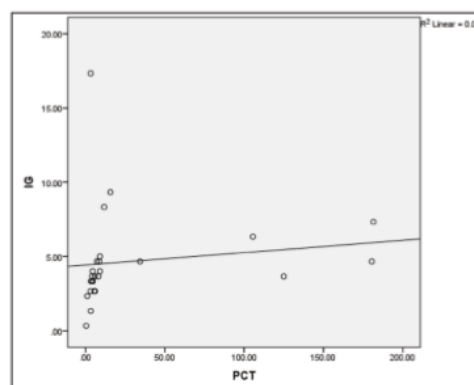


Figure 1b Correlation between IG and PCT ($p < 0.0001$; $r = 0.663$)

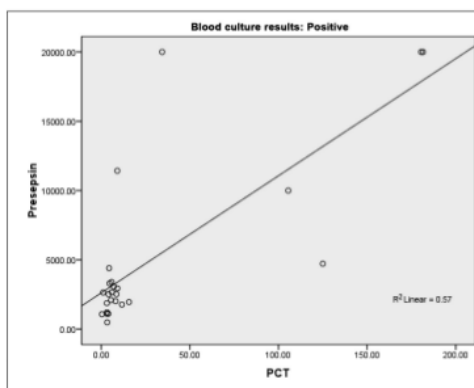


Figure 1c Correlation between presepsin and PCT ($p < 0.0001$; $r = 0.695$)

DISCUSSION

The incidence of sepsis in men is higher than in women. Pietropaoli et al. mentioned that the higher incidence of sepsis in men compared to women was caused by the presence of protective estrogen in the inflammatory mediator.⁸ The incidence of sepsis

in Dr. Soetomo General Academic Hospital in 2013 and 2014 showed that the prevalence of sepsis in men was higher than in women.³

The mean (\pm SD) age of patients was 51.24 (\pm 14.85) years; the majority of them were 51-60 years old. In 2013-2014, sepsis in Dr. Soetomo General Academic Hospital was dominated by patients aged 45-64. This indicates that sepsis incidence increases in the elderly.³ Immune system dysregulation, potential malnutrition, increased comorbidities, exposure to resistant pathogens in nursing homes, and increased dependence on invasive medical devices lead to high risk of infections and complications in elderly.⁹

This study obtained 21 patients with leukocytes $> 12,000$ per μL (84.0%). The natural immune response to extracellular bacteria is mainly through the mechanism of phagocytosis by neutrophils, monocytes, and tissue macrophages so that an increase in the number of leukocytes is accompanied by the presence of IG in septic patients.¹⁰

Most of the patients in this study had systolic pressure ≤ 100 mmHg (14 patients). This was due to dysfunction of endothel in sepsis caused by pro inflammatory cytokines, including increased leukocyte attachment, vasodilation, loss of endothelial defense function, and edema, resulting in a decrease in cardiac output, which in turn lead to a decrease in systolic pressure.¹¹

Blood culture is the gold standard for determining the presence of an infection, followed by an antibiotic sensitivity test so that the treatment of patients becomes more optimal.¹² In this study, positive blood cultures were found in 25 (31.65%) of 79 samples. A study conducted by Sarode et al. reported that 32 (25.8%) out of 124 sepsis patients had positive blood culture results.¹³ Blood cultures with false negative results may be the result of improper culture conditions of fastidious germs, technical errors (sampling process errors, less sample volume, the process of sending samples), or other inhibiting factors present in the blood sample.¹⁴ Negative blood cultures in this study may be due to non-bacterial sepsis conditions. In addition, the study subjects were taken from Dr. Soetomo General Academic Hospital, which is a referral hospital, meaning that most patients may have received prior antibiotic therapy.

In this study, the most common germs that cause bacterial sepsis are gram-positive bacteria (52.0%). This is in line with the study conducted by Basu et al., which reported that the most common cause was gram-positive bacteria, which was 119 of 221 patients.¹⁵

The results of this study indicated increased leucocyte CD64 in bacterial sepsis patients. Some

studies also showed that CD64 expression increased significantly in sepsis patients compared to healthy controls. CD64 is known as the Fc-gamma 1 receptor, which binds to monomeric IgG antibodies with high affinity in the process of phagocytosis intracellular microbial destruction.^{6,16} The CD64 cut-off value is 4.46%. We found two patients with leucocyte CD64 $< 4.56\%$. The low percentage of leucocyte CD64 may be due to the antibiotic therapy that the patient received beforehand. CD64 expression begins 4-6 hours after sepsis and stable for 48 hours.¹⁷

IG are useful markers for predicting infection. The cut-off value of IG is 0.5%. All IG results in this study were above the cut-off value. We found increasing IG percentage in bacterial sepsis patients. The research conducted by Van der Geest et al. also reported an increase in IG. They reported that the percentage of IG could increase in patients with infection and sepsis, especially in severe conditions. The results of IG examination in this study showed one patient with a fairly high percentage of IG (17.33%), which was in a patient with burns. A study stated that burn patients have increased IG and decreased neutrophil function.

In this study, we found increased levels of presepsin in bacterial sepsis patients. The concentration of presepsin in healthy people was very low compared to sepsis patients. The normal presepsin level is < 300 pg / mL.⁷ The cut-off value for sepsis diagnosis is 600 pg / mL. In this study, we found one subject with a presepsin level < 600 pg / mL. Low presepsin levels may be due to the antibiotic therapy that the patient received beforehand. Presepsin begins to increase 2 hours after the detection of bacteria or fungi, with a peak level after 3 hours, and a half-life of 4-5 hours.¹⁹ Several studies showed that presepsin has a sensitivity of 71-71% and a specificity of 70-86%.¹⁸

We found increasing PCT levels in bacterial sepsis patients in this study. The lowest level of PCT was 0.39 ng/mL, and the highest was 181.50 ng/mL, with a median value of 5.96 ng/mL. This is consistent with a study conducted by Schuetz et al., which stated that in septic patients, PCT levels increased by tens to hundreds of times. PCT levels > 0.5 ng/mL can be regarded as bacterial infections, whereas levels < 0.1 ng/mL can exclude bacterial infections.²⁰

We found one patient with PCT levels < 0.5 ng/mL. Low PCT levels may be due to the antibiotic therapy that the patient received beforehand because the sampling site is a referral hospital from various regions. PCT levels began to increase within 4 hours after sepsis, reached a peak after 6 hours and

stabilized within 8-24 hours. PCT values > 0.5 ng/mL are generally used as a cut-off for the diagnosis of sepsis, but 37.9% of patients with a diagnosis of bacterial sepsis had a PCT value < 0.5 ng/mL.⁶

PCT has been widely used for the diagnosis of sepsis, like other biomarkers such as erythrocyte sedimentation rate.^{6,21,22} Increased levels of PCT occur in a variety of severe infections and inflammation. PCT levels are found to be low in healthy people, but the levels increase in infectious, non-infectious and inflammatory diseases. These conditions lead to the release of pro-inflammatory mediators (e.g., IL-1 β , IL-6, and TNF- α) either through direct pathways (induced by lipopolysaccharide or toxins secreted by microbes) or cell-mediated responses. The proinflammatory mediator stimulates mono-cytes and induces calcitonin mRNA expression in non-neuroendocrine cells to release PCT, resulting in increased PCT levels.²¹

The results of the statistical analysis showed that there was no correlation between leucocyte CD64 and PCT in bacterial sepsis patients, although both of them increased. This is different from the research conducted by Angelina et al., which found a significant relationship between CD64 expression and PCT levels ($p = 0.036$) in patients with early neonatal sepsis.¹⁶ The percentage of leucocyte CD64 and PCT levels in bacterial sepsis patients in this study both increased, but the increase in the percentage of leucocyte CD64 was not in line with the increase in PCT levels. The absence of a correlation can be caused by differences in peak times of increased levels between leucocyte CD64 and PCT.

The sensitivity and specificity of CD64 in the study conducted by Sarode et al. were high with 96.77% and 100% respectively.¹³ Many other studies received 66% sensitivity and 65% specificity. Hoffmann stated that CD64 expression is not quite correct in distinguishing all sepsis patients from their severity, so it must be combined with medical history, physical examination, and other test results.¹⁸

There is a significant positive correlation between IG and PCT levels in bacterial sepsis patients. Research on the correlation between IG and PCT has never been done. The percentage of IG was reported to have a sensitivity and specificity of 89.2% and 76.4% respectively.¹⁰

The results of statistical analysis showed that there was a significant positive correlation between presepsin and PCT in bacterial sepsis patients. The ability of presepsin to predict bacteremia has been demonstrated in a study. Some studies showed that the area under the curve (AUC) from presepsin was not much different from PCT in predicting infection in the blood.

This study has several limitations, which are the unstandardized length of illness before patients' admission to Dr. Soetomo General Academic Hospital, the lack of attention regarding the administration of previous antibiotics, and the manual calculation of IG. All these factors could lead to bias.

CONCLUSION

This study concluded that there was no correlation between leucocyte CD64 and PCT, but there was a significant positive correlation between IG and presepsin and PCT in bacterial sepsis patients.

This is an initial study that analyzed the correlation of leucocyte CD64, IG, and presepsin with PCT levels in adult patients. Further research needs to be done by uniforming the sampling time and paying attention to the administration of previous antibiotics. IG testing should be done using an automatic tool. This is necessary to reduce bias and provide more reliable results.

The examination of IG and presepsin can be used as an alternative to PCT as a sign of sepsis. Currently, there is no single biomarker for the diagnosis of sepsis, so other additional examinations are still needed to diagnose sepsis. The examination of leucocyte CD64 needs to be carried out in further research before being it can be used as a marker of bacterial sepsis.

ETHICAL CLEARANCE

The study was approved by the Medical Ethics Committee of Dr. Soetomo Hospital, Surabaya, Indonesia (0330/KEPK/V/2018).

CONFLICT OF INTEREST

The authors declare that they have no competing interest regarding this manuscript.

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AUTHOR'S CONTRIBUTION STATEMENTS

Citra Novita, Yetti Hernaningsih and Anna Surgean Veterini conceived and designed the study method. Citra Novita performed the study. Citra Novita, Yetti Hernaningsih, Puspa Wardhani, and Anna Surgean Veterini analyzed the data. Citra Novita wrote the manuscript.

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